Customized Tissue Engineering Scaffold using Rapid Prototyping Technique in Rat Bone Augmentation

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In the last period of time, technology has advanced in such a manner that led to an important development of medicine. It happened due to the interchange of information between different areas of current interest such as engineering, computer science, electronics and medicine. There are already established the materials and technologies that combine all these areas: CAD-CAM technology, new imaging techniques as OCT, micro-CT, etc. Experiments were conducted on a sample of 10 rats 6 months aged. Bone augmentations were performed unilateral, in the medullary cavity of the femur. Femoral bones which had defects were examined using a multi-slice CT scanner (Somatom Sensation 64, Neuromed). Using CAD-CAM technology, scaffolds were perfectly matched with the bone defects. After analysis was performed, it was concluded that PGA / PLA is one of the best materials for bone regeneration. Evaluation and monitoring of bone augmentation are steps that can be studied by non-invasive methods of analysis, such as micro-CT.

Keywords: CAD-CAM technology, micro-CT, bone augmentation

As it is known, there are a lot of similarities between small animals, as rats, and the human body. This is the reason why, over time, the medicine was developed with the help of these animals. In dentistry is almost the same, the necesity of descovering new treatments and methods of treatment led to use animals for studies.

One of the biggest problems in the dental field is bone loss. It has a lot of causes, among the must important are osteoporosis and the teeth loss, caused by caries or periodontal diseases. Bone substance is needed in the absence of an adequate prosthetic field or in case of the insertion of dental implants.

In order to change the morphology and the form of the bone, the tissue augmentation is realised to the acceptor region. The material for augmentation can have a lot of origins, for example, it can be autogen (from the same individual), allogeneic (from the same species), xenogeneic (from another species), or synthetic. For better results, there is needed the addition of another biomaterial, the regeneration membrane. Regeneration membrans are resorbable or not materials that protect the bone defect restored after abbition.

When the process of augmentation has finnished, there is required a period of acceptance and healing of the bone.

In order to verify if the treatment will be a succes, there is a large market of technologies that can aid the dentist.

In this study the bone aumentations were made on rats femur. The analisis of results after the healing process was made using micro-CT technology.

Micro-CT specimen scanners are indicated because of the high-resolution, giving information about geometry, density and microarchitecture of mineralized tissues, especially bones, or teeth. Particularly important is that this technology provides the 3D representation of the analyzed structure. In understanding the 3D complex, the orientation, the connectivity of trabeculae and the shape, are the elementary parameters. MicroCT is helpful in monitoring the bone changes in several pathophysiological cases.

The latest generation micro-computed tomography (micro-CT) can provide images with resolution of less than 10 μm.

However, there are only few methods dedicated to in vivo and ex vivo noninvasive evaluations. The common investigation methods imply sectioning analysis and can not be taken into consideration in clinical practice. Also these methods are limited to the dimensions of the cutting devices. One of the most important advantage of this micro-CT method is that is very efficient, being a non-invasive research method [1], and because of its potential for use in vivo evaluation.

Micro-CT was successfully applied for advanced characterization in bone [2, 3] and bone tissue engineering [4,5], cardiology [6] muscular dystrophy research [7] and other medical and technological fields [8, 9]. Micro-CT was also employed for tooth and relative problematic non-distructive investigations [10].

Micro-CT is similar to conventional computer tomography (CT) usually employed in medical diagnosis and in industrial applied research. Unlike these systems, which typically have a maximum spatial resolution of about 0.5 mm, micro-CT is capable of achieving a spatial resolution of up to 0.3 microns [11, 14, 15].

Computer-aided design (CAD) and computer-aided manufacturing (CAM) systems are capable to manipulate the three-dimensions (3D) using computed tomography (CT) images of bone, using a virtual reality force feedback device. An exceptional innovation in engineering is Rapid Prototyping (RP) that has been inserted in the medicine field, to create models that provide both tactile and visual

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information. CAD/CAM and RP techniques were adapted in order to allow the generation of scaffolds for cell delivery that are custom-made to fit into given bone defects. Craniomaxillofacial bone has the characteristic of being an irregular bone, having a subtle 3D structure and individualized repair of bone defects, which is very important. This is the reason why CAD-CAM, laser scanning and RP technologies have been applied in cranio-maxillofacial surgery. The scaffolds used must fit into the anatomical defect and must have sufficient mechanical integrity and also a controllable degradation rate. The synthetic biodegradable polymers poly (lactic acid) (PLA), poly (glycolic acid) (PGA) and poly (lactic-co-glycolic acid) (PLGA) represented more interest in tissue engineering because of their excellent biocompatibility, uniform quality, ease of fabrication into desired anatomical forms and controllable degradation timescales compared to natural macromolecules [12].

There must be available cells for bone tissue engineering in large numbers and must be able to describe the cartilage and bone phenotypes. Bone marrow stem cells (BMSCs) can differentiate into osteogenic, adipogenic and fibroblastic cells. The method for collect these cells is well established, BMSC lines being readily propagated for long periods without losing their potency.

Experimental part

Biological material for the in-vivo experiments consist in 10 Wistar rats, 6 months old from the Animal House of the "Pius Branzeu" Center for Laparoscopic Surgery and Microsurgery with an average weight of animals at surgery of 300g, which were quarantined two weeks before starting of the surgical experiments. The bone augmentation materials were implanted unilaterally in the medullary cavity of the femur of each animal. The distribution between sides will be equal. For the entire experimental period the animals will be kept two or three in a cage with an unlimited supply of fresh water and rodent pellets. The experimental protocol will submit for approval to the Ethical Committee of University of Medicine and Pharmacy, Timisoara and the Local Veterinary Authority.

The femurs with custom made defects were examined using a multi-slice CT scanner (Somatom Sensation 64, Neuromed). In the case of Somatom Sensation 64, the spatial resolution of an image is lower than 0.4 mm, and the acquired volume unit (voxel) has the same size for all 3 dimensions (under 0.4 mm for the x , y and z axes). Obtaining such a resolution is possible due to the technical parameters offered by this machine and, particularly, the high rotation speed of the tube (330 ms) and the technical ability of the STRATON tube to generate two fascicles of X-rays which intertwine, generating the spatial resolution of 0.3 mm. The raw data were exported to Digital Imaging and Communications in Medicine (DICOM) 3.0 format files. A 3D digital model of the femurs (without soft tissue and cartilage) was then reconstructed slice by slice and compiled into a solid geometric model in STL format. The STL files were then imported into a rapid prototyping printer system that enables 3D printing of the scaffolds.

After clinical examination, only the animal considered healthy were used. Before surgery X-ray exams evaluation was performed for the femoral and tibia region for excluding eventually pathology at places of bone material augmentation insertion.

Anesthesia of the animals is achieved with Isofluran in concentration of 5% and O_2 at 1L/min for induction in the anesthesia chamber and after the animal is connected throw a facemask to an open breath circuit of anesthesia which inflows Isofluran at 1% and O_2 at 1L/min. The hindquarter on the experimental side is shaved and the entire dorsal aspect of the animal is disinfected.

Surgery is performed under sterile conditions. Access to the periosteum is made throw a 3 cm. longitudinal incision of the skin on the lateral part of the thigh region, in the cranial third, followed by the blunt dissection of the quadriceps muscle with exposure of proximal diaphysis of femoral bone. All the maneuvers are performed under the microscope and the tissues are handled with microsurgical instruments to prevent as much as possible the tissue distruction. Group 1 (20 animals) will have a circular defect perpendicular to the bone of approximately 3mm in diameter and 2 mm deep using Surgic XT Plus, a surgical motors which provide speed and high torque accuracy for maximum safety during operation. Group 2 (20 animals) consist in making of a V-shaped defect with the top of "V" oriented transversal on the length of the bone. Group 3 (20 animals) consist in creating of a longitudinal bone defect in 3 mm long and 1 mm wide. Bone defects are performed with VarioSurg, which is a powerful ultrasonic saw. The ultrasonic approach to bone surgery reduces heat generation in order to minimize osteonecrosis and avoid damage to surrounding soft tissue.

All rats are euthanatized (Thiopental overdose) at 84 days after surgery. The tight is exposed through an extended lateral incision. Soft tissues were removed with preservation of the periosteum. After exarticulation of the knee and hip joints, the femurs are removed and the specimens with new bone formation region will be gross examined, emphasizing the changing, after that is being prepared, together with bone structure for micro-CT evaluation. The peak energy for the system was 90kVp. The resolution was 10 microns and the scanning time was 2 h for each considered sample.

Results and discussions

With the technology of CAD/CAM and Rapid Prototyping, the scaffolds are perfectly matched for the defects made on the femurs. There were taken into consideration both, studies that evaluated the suitability of various materials in the construction of CAD/CAM scaffolds, another histological studies focusing on BMSC seeding in scaffolds have shown that PGA/PLA is one of the best materials disposable for the regeneration of new bone and cartilage.

However, sculpting the external volume of the scaffold and generating strategies for its RP using PGA/PLA are significant requirements. There was described a new method to tailor-make PGA/PLA mandibular condylar scaffolds for bone and cartilage regeneration that guarantees an excellent morphological alteration and accuracy.

It was generated a physical replica of the mandible with 3D CT data using CAD/CAM and RP, being used as the positive model in the negative mould technique to prefabricate a PGA/PLA scaffold in the shape the same as the mandibular condyle. To analyze the accuracy of the copy thus obtained, was used a laser scanning system.

By generalizing, if the difference could be seen in <90% of the face after simulated scaffold placement, this was doomed to be a reliable reproduction; in addition, when aligned, maps of the merged scans revealed that, on average, 90% of the created composite facial scans correlated to the original with an error up to 0.85 mm, that was considered to be clinically acceptable. As respect of bone reconstruction of craniomaxillofacial features, the tolerance levels reached in our results are more acceptable. A comparison between the copy and the original morphology confirmed the efficacy of this method for accurate reconstruction of craniomaxillofacial characteristics. Also, synthetic absorbable implants that are made of materials such as elastic PGA/PLA can be readily adapted to the form of a desired defect. Thanks to their resilience, these materials may not sufficiently resist at static or dynamic stresses[13].

Whereas, each material has its advantages and disadvantages, it is required at least to accomplish its specified objectives. PGA and PLA, for example, are degradable, and their degradation product is weakly acidic. The acid–base neutralization reaction that occurs in certain places during degradation can prevent aseptic inflammation, maintain acid–base balance and promote osteogenesis. Zhou demonstrated that physiological repair of deficiencies in articular cartilage and the corresponding subchondral bone was accomplished using autologous BMSCs and PGA/PLA polymers. This study proves that BMSCs dispersed throughout a PGA/PLA scaffold in "in vitro" culture, showing that PGA/PLA have good cellular compatibility. Also, BMSCs have multi-lineage differentiation potential, especially for osteogenic and chondrogenic differentiation, making them ideal seeding cells for tissue engineering.

Conclusions

Evaluation of the bone grafting material/bone interface with noninvasive methods such as micro-CT could act as a valuable procedure that can be used in the future for usual research procedures. The rapid prototyping technologies tend to become the future alternative for the custom made scaffolds for bone augmentation.

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References

1.CHINELATTI MA, CORONA SA, BORSATTO MC, RIBEIRO LF, ROCHA RA, PALMA-DIBB RG. Analysis of surfaces and adhesive interfaces of enamel and dentin after different treatments. J Mater Sci: Mater Med 2007;18:1465-70.

2.TAVELLA S, RUGGIU A, GIULIANI A, BRUN F, CANCIANI B, MANESCU A, et al. Bone turnover in wild type and pleiotrophin-transgenic mice housed for three months in the international space station (ISS). PLoS ONE 2012;7(3):e33179.

3.CANCEDDA R, CEDOLA A, GIULIANI A, KOMLEV V, LAGOMARSINO S, MASTROGIACOMO M, et al. Bulk and interface investigations of scaffolds and tissue-engineering bones by X-ray microtomography and X-ray microdiffraction. Biomaterials 2007;28:2505-24.

4. GIULIANI A, FIORI F, MANESCU A, VLADIMIR SK, RENGHINI C, RUSTICHELLI F. Synchrotron radiation and nanotechnology for stem cell research. In: Gholamrezanezhad A, editor. Stem Cells in Clinic and Research. Rijeka, Croatia: Published by InTech; 2011.,ISBN 978-953-307-797-0.

5. GIULIANI A, FRATI C, ROSSINI A, KOMLEV VS, LAGASTA C, SAVI M, et al. High-resolution X-ray microtomography for three-dimensional imaging of cardiac progenitor cell homing in infracted rat hearts. J Tissue Eng Regen Med 2011;5(August(8)):e168-78.

6. FARINI A, VILLA C, MANESCU A, FIORI F, GIULIANI A, RAZINI P, et al. Novel insight into stem cell trafficking in dystrophic muscles. Int J Nanomed 2012;7:3059-67.

7. POZDNYAKOVA A, GIULIANI A, DUTKIEWICZ J, BABUTSKY A, CHYZHYK A, ROETHER JA, et al. Analysis of porosity in NiTi SMA's changed by secondary pulse electric current treatment by means of ultra small angle scattering and micro-computed tomography. Intermetallics 2010;18:907-12.

8. MANESCU A. DI GREGORIO GM, GIRARDIN E, CALBUCCI V, ANGHELONI G, CARTA P, et al. Defect analysis on optical waveguide arrays by synchrotron radiation microtomography. IEEE Trans Dev Mater Reliab 2011;11(4):548-50.

9. GIULIANI A, KOMLEV V, RUSTICHELLI F. Three-Dimensional Imaging by Microtomography of X-ray Synchrotron Radiation and Neutrons. Innovative Technological Materials. Berlin, Heidelberg: springer-Verlag; 2010,ISBN 978-3-642-12058-9. P. 123.

10. SWAIN MV, XUE J, State of art of Micro-CT applications in dental research. Int J Oral Sci 2009;1(December(4)):177-88.

11.SCHNEIDER CA, RASBAND WS, ELICEIRI KW. NIH Image to ImageJ: 25 years of image analysis. Nat Methods 2012;9:671-5.

12. ROMINU, M., MANESCU, A.,SINESCU, C., NEGRUTIU, M.L., TOPALA, FL., RONINU, R.O., BRADU,AJACKSON, D. A., GIULIANI, A., PODOLEANU. A. Gh., Zirconia Enriched dental adhesive: A solution for OCT contrast enhancement. Demonstrative Study by synchrotron radiation microtomograply, Dental Materials 30 (2014) 417-423, ISSN 0109-5641.

13. DONG HAN, JIASHENG DONG, DE JUN CAO, ZHE-YUAN YU, HUA XU, GANG CHAI, SHEN GUO-XIONG AND SONG-TAO AI (2011). Usage of Rapid Prototyping Technique in Customized Craniomaxillofacial Bone Tissue EngineeringScaffold, Advanced Applications of Rapid Prototyping Technology in Modern Engineering, Dr. M. Hoque (Ed.), ISBN:978-953-307-698-0, InTech, Available from: http://www.intechopen.com/books/advanced-applicationsof-rapid-prototyping-technology-in-modern-engineering/usage-of-rapid-prototyping-technique-in-ustomizedcraniomaxillofacial-bone-tissue-engineering-scaff

14. RUSU,L.C., ARDELEAN, L., NEGRUTIU, M., DRAGOMIRESCU, A., ALBU, M., GHICA, M., TOPALA, F.,A PODOLEANU, A., SINESCU, C., Rev.Chim.(Bucharest), **62**, no. 8, 2011,p. 841

15. RUSU, L.C., BORTUN, C-M., HOINOIU,B., NEGRUTIU, M-L., ARDELEAN, L., PODARIU, A.C., SINESCU , C., Rev. Chim.(Bucharest), **65**, no. 12, 2014, p. 1495

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